# THE SANITARY QUALITY OF MARKET OYSTERS

BY

THOMAS HENRY LORD

B.S., Massachusetts State College, 1936

M.S., University of Illinois, 1938

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
BACTERIOLOGY IN THE GRADUATE SCHOOL OF
THE UNIVERSITY OF ILLINOIS, 1941

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## THE SANITARY QUALITY OF MARKET OYSTERS

### 1. INTRODUCTION:

The oyster had been incriminated in the spread of disease at an early period in the history of the pure food movement. The American Public Health Association and the United States Public Health Service have been working in this country to improve the status of oysters to the point where they may be consumed without danger of infection by intestinal pathogens. Most of the work on the sanitary quality of shellfish has been done on oysters from coastal markets. This investigation was pursued to determine the sanitary condition of raw shucked oysters on a typical mid-western market.

### 2. REVIEW OF THE LITERATURE:

A review of the literature is presented covering the following topics: The oyster as a food of man, the economic aspects of the oyster industry, the oyster as an animal, oyster culture, fattening of oysters, floating of oysters, hibernation and storage of oysters, purification, shucking and packing for shipment, adulteration of oysters, nutrition of oysters, effects of pollution on oysters, coliform organisms and oysters, *Eberthella typhosa* and oysters, typhoid fever outbreaks caused by oysters, the bacterial flora of live oysters, spoilage of oysters, chemical methods of determining purity of oysters, and the development of a standard method for the bacteriological examination of oysters.

### 3. EXPERIMENTAL TECHNIQUE:

Samples of oysters purchased on the open retail market were used throughout the investigation. Two hundred grams of sample were weighed into a sterile mixing cup of a Steven's mixer. Two hundred milliliters of sterile 1 per cent NaCl solution were added. The cup was covered with the sterile cover and the sample disintegrated for 5 minutes. Dilutions of 1:10 and 1:100 were made using sterile 1 per cent NaCl solution.

The following liquid media were inoculated in parallel: Standard lactose broth, Eijkman broth, brilliant green bile 2 per cent lactose broth, brilliant green bile 5 per cent lactose broth, formate ricinoleate broth, methylene blue-brom cresol purple broth, purple bile salt broth, gentian violet lactose bile broth, crystal violet lactose broth, fuchsin lactose broth, phenol red broth, Duponol

Wa tryptose lactose broth 1:5000. The following plating media were used: Violet red bile agar, brilliant green lactose bile agar, purple lactose agar, buffered purple lactose agar, plain nutrient agar, and tryptone glucose agar with 1 per cent sodium chloride.

The liquid media were inoculated with 2 milliliters of macerated sample, and 1 milliliter of 1:10 and 1:100 dilutions. Plating media were inoculated with 2 milliliters of macerated sample and decimal dilutions to 1:100,000. All incubation was carried out at 37°C.(98.6°F.) unless otherwise stated. Detection of *Escherichia coli* was carried out according to the 1940 revision of Standard Procedure for the Bacteriological Examination of Shellfish and Shellfish Waters, using brilliant green bile 2 per cent lactose broth, Endo's agar, and Koser's citrate medium for the confirmation procedure. Results of examinations for coliform group and *Escherichia coli* were recorded in terms of "Most Probable Number per 100 grams of sample."

A pH determination was made on the free liquor and macerated sample. The appearance and odor of the sample were noted.

4. RESULTS:

1. Purple bile salt broth of the following composition:

| | | |
|---|---|---|
| Lactose | 10.7 | grams |
| Peptone | 21.4 | grams |
| Ox-gall | 5.33 | grams |
| Brom Cresol Purple | 0.064 | grams |
| Water | 1,000.0 | milliliters |

was the most efficient medium for detection of *Escherichia coli* in fresh shucked oysters. The media used ranked as follows in the order of decreasing efficiency: Purple bile salt broth, formate ricinoleate broth, phenol red broth, brilliant green bile 5 per cent lactose broth, brilliant green bile 2 per cent lactose broth, gentian violet lactose bile broth, standard lactose broth, Duponol Wa tryptose lactose broth 1:5,000, crystal violet lactose broth, fuchsin lactose broth, methylene blue-brom cresol purple broth, and Eijkman broth incubated at 45.5°C.

2. The Standard Procedure for Shellfish Examination can be shortened to 24 hours' incubation of the purple bile salt presumptive medium, followed by 48 hours' incubation of brilliant green bile 2 per cent lactose broth, and completed on Endo's agar (or eosin-methylene blue agar) and Koser's citrate medium for 24

hours each. This practice will be over 94.6 per cent efficient in detecting *Escherichia coli* in oysters. If time is not an important element the longer Standard Method can be used with an efficiency approaching 100 per cent.

3. Direct plating for enumeration of *Escherichia coli* did not prove to be satisfactory when using: Buffered purple lactose agar, Endo's agar, violet red bile agar, and brilliant green lactose bile agar. Violet red bile agar was the most satisfactory medium of the five used and can be used to obtain an idea of the trend of numbers of *Escherichia coli*-like organisms in oysters.

4. A temperature of incubation of 20°C.(68°F.) gives 2 to 17 times higher counts on plain nutrient agar than a temperature of incubation of 37°C.(98.6°F.).

5. Tryptone glucose agar with 1 per cent sodium chloride is favored over plain nutrient agar for total aerobic counts at 37°C. (98.6°F.) because fewer pin-point colonies are produced. Counts on both media at 37°C. (98.6°F.) are of the same magnitude.

6. "Fresh" oysters had pH values above 5.4. "Sour" oysters had pH values below 5.4. A difference of 0.2 pH was found between the value of the free liquor and the macerated sample, the pH of the latter being lower.

7. Approximately 60 to 65 per cent of the 75 fresh shucked oyster samples examined had Most Probable Numbers of 2500 (equivalent to a "score" of 50) or higher. Eleven per cent of the samples would have passed a strict standard of not over 100 *Escherichia coli* per 100 grams. Twenty-seven per cent would have passed a lenient standard of not over 1000 *Escherichia coli* per 100 grams.

8. Examination of 15 samples of frozen shucked oysters revealed an almost sterile product with insignificant numbers of *Escherichia coli* (80 per 100 grams for the highest sample). The pH values of frozen oysters ranged from pH 4.38 to pH 5.4. The highest aerobic count obtained on tryptone glucose agar with 1 per cent sodium chloride was 28,000 bacteria per 100 grams. The average count was 710 bacteria per 100 grams.

## 5. CONCLUSIONS:

The writer has found purple bile salt broth a more efficient medium to use in the presumptive test and prefers its use to

standard lactose broth. If purple bile salt broth is substituted in the Standard Procedure, the analysis can be shortened to a 24-hour incubation period in the presumptive test, followed by routine confirmation with an efficiency of 94.6 per cent or better.

Direct plating for *Escherichia coli* is too variable to be recommended.

Total aerobic plate counts on tryptone glucose agar with 1 per cent sodium chloride at 37°C.(98.6°F.) is recommended for indicating gross mishandling and/or improper refrigeration of the product as reflected in high numbers of bacteria.

The pH determination has value in detecting spoilage, particularly when foreign odors are present.

The writer prefers the expression of results in terms of "Most Probable Numbers" rather than as an arbitrary "score" to make the method of expression of results comparable to that used for other foods.

On the basis of a proposed standard of less than 1000 *Escherichia coli* per 100 grams, 27 per cent of the samples would be considered satisfactory. The frequent high plate counts and low pH determinations show that the fresh shucked oysters on a typical mid-western market have been subjected to improper handling, inadequate refrigeration, or both.

The sanitary quality of frozen shucked oysters was excellent. The samples were practically sterile and had very low Most Probable Numbers of *Escherichia coli*, indicating a clean, properly-cared-for product.

## VITA

Thomas Henry Lord was born June 21, 1914, at Somerville, Massachusetts. He received his elementary and high school training from the Arlington, Massachusetts, public schools.

In June, 1932, he was graduated from the Arlington (Massachusetts) High School, the recipient of a Blake award for high scholarship. In September of that same year he enrolled in the Massachusetts State College at Amherst, Massachusetts, to pursue a major course in Bacteriology and minor courses in Chemistry and Zoology. During his undergraduate training he was cited on the Dean's Honor List. He received his diploma of Bachelor of Science in Bacteriology in June, 1936.

In September, 1936, he began graduate study at the University of Illinois, Urbana, Illinois, while holding the position of half-time assistant in the Department of Bacteriology. The degree of Master of Science in Bacteriology was conferred upon him in February, 1938. During the academic year 1940-41 he was promoted to the position of full-time assistant in the Department of Bacteriology.

He has just been appointed Instructor in Bacteriology at the Kansas State College, Manhattan, Kansas, beginning September 1, 1941.

He is a member of Kappa Epsilon, Gamma Alpha Scientific Fraternity, Phi Sigma Society, Society of Illinois Bacteriologists, and an active member in the Society of the Sigma Xi.